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Identification and Characterization of White Grape Varieties Autochthonous of a Warm Climate Region (Andalusia, Spain)

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Abstract: The high sensitivity of one of the most important crops in the world, such as vine (*Vitis vinifera* L.), to particular changes caused by the phenomena associated with global warming, is encouraging the wine industry to place value on grape varieties that are autochthonous to each production area. These are generally conserved in germplasm banks and may pose a useful tool to counteract the effects of climate change. In order to determine the actual resource that such varieties constitute, this research has carried out a genetic identification, a morphological characterization, and an analysis of the grape musts obtained from four autochthonous varieties (Cañocazo, Castellano, Mantúo de Pilas, and Palomino Fino). This genetic analysis has allowed the identification of autochthonous varieties with different genotypes. However, all of them had similar phenotypic characteristics in terms of high hair density in adult leaves. With respect to the physicochemical composition of the musts, significant differences have been observed between the autochthonous varieties, with respect to the control variety of Palomino Fino. Nevertheless, all of them have exhibited an adequate physicochemical composition to produce quality white wines. For all of the above reasons, these local varieties should be considered suitable for cultivation in areas with warmer and drier climates, such as Andalusia (Spain).

Keywords: *Vitis vinifera*; autochthonous variety; simple sequence repeat analysis; warm climate

1. Introduction

The so-called area known as Marco de Jerez, located in the south of the Iberian Peninsula, is one of the most important wine-growing regions in Spain, which reached its fullness and international recognition during the 19th century [1]. However, the wines produced in this area have evolved throughout history because of different biological and political circumstances. From a viticultural point of view, the invasion of phylloxera in the area in 1894 caused the loss of a large part of the Jerez vineyards, which had to be replanted [2]. This led to a significant loss of vine varieties. Clemente and Rubio [3], at the beginning of the 19th century, described 43 vine varieties that were cultivated in the Marco de Jerez vineyards before the phylloxera outbreak. After the replanting of the vineyards to deal with the plague, the number of varieties cultivated dropped significantly. Fernández de Bobadilla [4] quotes among other replanted vines: Palomino Fino, Pedro Ximénez, Cañocazo, and Albillo as classic varieties, Garrido, Perruno, Mantúo, and Beba as secondary varieties, and Moscatel and Tintilla de Rota as special varieties. Subsequently, severe regulations were approved and the vine varieties that were authorized for wine production were restricted [5]. Likewise, in the second half

of the 20th century, based on productivity criteria, clone plant material was introduced in the new Palomino Fino plantations. This caused a loss of genetic resources from this variety. Consequently, only three white grape varieties are currently grown in Marco de Jerez for the production of Sherry wines: Palomino Fino, Pedro Ximénez, and Moscatel, although the last two are grown at a very small scale [6]. For this reason, in order to preserve the biodiversity in the *Vitis vinifera* species, and to safeguard the different autochthonous varieties from each zone, grapevine germplasm collections, or banks, were created [5,7–10].

At present, these autochthonous vine varieties, which have been conserved in the germplasm banks, can be considered a valuable genetic resource for addressing one of the most important challenges that the global wine sector faces: global warming [5]. Each variety has its own specific genotype, morphology, and content in its secondary metabolites that make it unique [11]. All of these elements explain the adaptation of vine varieties to different climates, or environments, and the physicochemical properties that their berries have [12–15]. There are numerous works related to the genetic and morphological characterization of autochthonous vine varieties using Simple Sequence Repeat (SSR), or microsatellites markers and ampelographic descriptors [16–18]. However, these studies do not include data on the physicochemical compositions of their musts, which are decisive to determine their oenological potential and the adaptation capacity of these varieties to the climatic conditions in the area. In addition, the studies on the identification and characterization of the agronomic and oenological behavior of a particular vine variety are an essential requirement when it comes to applying for its inclusion in the register of authorized varieties.

For all these reasons, the main objective of this work focuses on the identification and characterization of white autochthonous grape varieties grown in a warm climate region (Andalusia, Spain). Its morphological and molecular characterization could contribute to the detection of new synonyms, homonyms, or false attributions. On the other hand, the analysis of their musts could contribute to producing new white wines in warm climate areas.

2. Materials and Methods

2.1. Grapevine Material

Three autochthonous vine varieties have been analyzed: Cañocazo (CÑ), Castellano (CS) and Mantúo de Pilas (MP). Palomino Fino (PF) has been employed as the control variety, since it is the most commonly cultivated autochthonous variety in the Marco de Jerez region (Andalusia, Spain) [19]. A total of ten plants from each variety were selected for the study (2016–2017), following Santesteban et al. [20] criteria, in order to minimize the intrinsic variability of samplings. For this purpose, the trunk cross sectional area (TCSA) of 40 vines was measured at a 30 cm height using a digital Vernier Caliper Maurer 93110 (Padova, Italy). The 10 plants marked as subjects were selected for presenting a TCSA value close to the mean ± 10%. All these varieties were planted on the same date and were located on the same plot (latitude 36° 41′ 10″ N; longitude 6º 08′ 10″ W; 20 m above sea level), in the municipality of Jerez de la Frontera (Cadiz, Spain). The plot has a limestone soil, a plantation surface of 2.30 × 1.15 m, and a double Guyot training system. No fertilization or irrigation treatments were applied during the studied years, and different conventional phytosanitary products were applied to obtain ripe and sound grapes. Supplemental Figures S1a–c and S2a–c show the historical temperature, humidity, precipitation, and solar radiation during the period between the veraison and the harvest date (from July to September) in the two years studied respectively.

Only for genetic characterization (SSR analysis), four other reference varieties planted in the same plot (Cabernet Sauvignon (CSV), Chardonnay (CH), Muscat a Petits Grains Blancs (MPGB), and Pinot Noir (PN)) were included in order to compare the genotypes obtained with those in the databases, to confirm the identity of the variety analyzed.

2.2. Simple Sequence Repeat (SSR) Analysis

A total of 22 nuclear microsatellite loci were employed to perform the varietal identification following the methodology proposed in recently published papers [21]. A DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was employed to carry out the DNA extraction. PCR amplifications were performed using a 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA), and the amplified products were separated by capillary electrophoresis, using an automated sequencer ABI PRISM 3130 (Applied Biosystems, Foster City, CA, USA). Fluorescent labelled fragments (6-FAM, VIC, PET, and NED) were detected and sized using GeneMapper v. 3.7, and fragment lengths were assessed with the help of internal standards GeneScan-500 LIZTM (Applied Biosystems, Foster City, CA, USA). The comparison of the SSR obtained was performed using a microsatellite toolkit v. 9.0 software [22]. Lastly, the microsatellite genotypes obtained after the analysis were compared to the genetic profiles given by Lacombe et al. [23], and to the data contained in several genetic databases [24–27] and scientific research.

2.3. Ampelographic Characterization

Three autochthonous varieties (Cañocazo (CÑ), Castellano (CS), and Mantúo de Pilas (MP)), and the control variety Palomino Fino (PF) were characterized ampelographically. At least ten young shoots, young and mature leaves, flowers, bunches, and berries from each variety were described using Benito et al. [28] criteria, and 36 descriptors, according to the International Organization of Vine and Wine's descriptor list [16] (14 priority descriptors for primary descriptions plus 22 additional descriptors). Eight of those descriptors correspond to the branches, 19 to the leaves, one to the inflorescence, four to the bunches, and four to the berries. Samples from two consecutive crops of all the varieties were described by five ampelographers with varied expertise knowledge. The modal value for each descriptor was selected as the final descriptor.

2.4. Physicochemical Analysis

For the physicochemical characterization, 5 kg of berries of each variety (500 g per plant) were harvested on the date recommended by the winery. All the samples were harvested at the same time since the varieties were planted on the same plot and were therefore processed when the control variety Palomino Fino was harvested. The sugar content (°Bé), total acidity (TA), pH, tartaric acid, malic acid, glycerine, oxidative index, yeast assimilable nitrogen (YAN), and the concentration of cations potassium, calcium, magnesium, iron, copper, and sodium were determined in the musts of the four varieties that were studied for two consecutive years. All of the analyses were carried out in triplicate.

°Bé was determined using a calibrated Dujardin–Salleron hydrometer (Laboratories Dujardin-Salleron, Arcueil Cedex, France). Total acidity (TA) was assessed following the International Organization of Vine and Wine (OIV) reference method [29]. The pH was measured using a digital pH-meter CRISON-2001 (Crison, Barcelona, Spain), equipped with a combined electrode with automatic temperature compensation. Organic Acids (tartaric and malic acid) were assessed using an ionic chromatograph (Metrohm 930 Compact IC Flex) with a conductivity detector, following the conditions proposed by Sancho-Galán et al [30]. Yeast assimilable nitrogen (YAN) was determined according to the described formal method [31]. The glycerine content of the samples was determined by means of an enzymatic kit (Biosystems, Barcelona, Spain) and the colorimetric measurement of the enzymatic reaction in a HITACHI UV-Vis spectrophotometer (Pacisa y Giralt S.L, Madrid, Spain). All the samples were previously filtered through a 0.45 µm nylon syringe filter (FILTER-LAB, Barcelona, Spain) for chromatographic and spectrophotometric analysis. To calculate the oxidative index, the musts were measured at a wavelength of 420 nm. The musts were then incubated at 45 °C for 5 days, and after this time, the absorbance was again determined at 420 nm. The oxidative index was calculated according to the equation $((Abs_{end} - Abs_{beginning})/Abs_{beggining}) \times 100$ and expressed as a percentage.

To determine the cation content in the musts, 20 mL of the samples were first incinerated in a Carbolite ELF 11/148 furnace (Sigma Aldrich, Saint Louis, United States) at 500 °C for two hours. Once the ashes were obtained, they were digested acidically using nitric acid, following the protocol proposed by the Association Française de Normalisation (AFNOR) [32]. All the cations were determined by atomic emission spectroscopy by inductively coupled plasma in an Iris Intrepid ICP-AES (Thermo Scientific, Waltham, United States).

2.5. Statistical Analysis

Means and standard deviations were calculated and significant differences were evaluated by one-way ANOVA and Bonferroni's multiple range (BSD) test with a p-adjust < 0.05 (GraphPad Prism version 6.01 for Windows, GraphPad Software, San Diego, CA) statistical package. Principal component analysis (PCA) was performed using the statistical computer package SPSS 23.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Simple Sequence Repeat (SSR) Analysis

The genotypes obtained after the analysis of the autochthonous varieties and reference varieties with 22 microsatellites loci are shown in Table 1. The three microsatellite profiles obtained for each one of the autochthonous varieties have been matched with known varieties or "prime names" according to the *Vitis* International Variety Catalogue (*V*IVC). In addition, the different genotypes have been compared to the genotypes published in the databases that are kept at Rancho de la Merced Germplasm Bank [7,25], the El Encín Germplasm Bank [26,27], and other European databases [23], in order to establish new synonyms.

Table 1. Genetic profiles of the autochthonous and reference varieties at 22 microsatellite loci. Allele sizes are given in base pairs.

	Autochthonous Variety								*Reference Variety*							
Variety Code	**CÑ**		**CS**		**MP**		**PF**		**CSV**		**CH**		**MPGB**		**PN**	
Microsatellite Locus																
VVIB01	291	307	291	307	307	307	291	307	291	291	289	295	291	295	289	295
VMC1b11	184	188	184	184	184	188	184	188	184	184	166	184	184	188	166	172
VMC4F31	188	206	168	176	184	190	176	206	174	178	174	180	168	206	174	180
VVMD5	232	234	220	224	224	232	226	238	228	236	232	236	226	234	226	236
VVMD7	240	246	236	246	244	246	236	246	236	236	236	240	232	246	236	240
VVMD21	249	255	243	265	243	249	243	249	249	257	249	249	249	265	249	249
VVMD24	209	209	209	211	209	209	209	209	209	217	209	219	213	217	215	217
VVMD25	240	252	252	252	238	252	240	240	238	246	238	252	240	246	238	246
VVMD27	186	194	182	182	182	182	186	194	176	190	182	190	180	194	186	190
VVMD28	236	250	246	246	246	248	238	250	236	238	220	230	248	270	220	238
VVMD32	254	270	270	270	270	270	254	256	238	238	238	270	262	270	238	270
VVIH54	166	166	166	168	166	168	166	166	166	182	164	168	166	166	164	168
VVIN16	153	153	151	151	151	153	151	151	153	153	151	151	149	149	151	159
VVIN73	264	264	264	264	264	264	264	264	264	268	264	266	264	264	264	266
VVIP31	180	190	176	176	176	190	188	190	190	190	180	184	184	188	180	180
VVIP60	318	326	322	322	318	326	318	322	306	314	318	322	318	318	318	320
VVIQ52	85	89	85	89	85	89	85	85	83	89	83	89	83	83	89	89
VVS2	142	144	142	142	131	142	131	144	137	151	135	142	131	131	135	151
VVIV37	163	177	163	167	161	161	163	167	163	163	153	163	163	165	153	163
VVIV67	358	372	366	375	372	375	364	366	364	372	364	372	364	375	364	372
VrZAG62	187	203	187	193	187	193	187	193	187	193	187	195	185	195	187	193
VrZAG79	236	246	236	258	242	248	250	260	246	246	242	244	250	254	238	244

CÑ: Cañocazo. CS: Castellano. MP: Mantúo de Pilas. PF: Palomino Fino. CSV: Cabernet Sauvignon. CH: Chardonnay. MPGB: Muscat a Petits Grains Blancs. PN: Pinot Noir.

3.2. Ampelographic Characterization

The results of the morphological description are shown in Table 2. All the varieties presented different phenotypes. The main morphological differences in the leaves were observed in the variety Mantúo de Pilas, which presented a very high density of prostate hairs between main veins on the lower side of the blade in young leaves (OIV 053), and a very high density in prostate hairs on the main veins on the lower side of the blade in adult leaves (OIV 086). OIV 233 descriptor refers to the shape of the berry, and was the most discriminating descriptor among the four varieties characterized, with different shapes being observed for each of the varieties.

Table 2. Modal values for the International Organization of Vine and Wine (OIV) ampelographic descriptors observed in the four varieties analyzed during two consecutive years.

Code	Descriptor	CÑ	CS	MP	PF
OIV 001	Young shoot: opening of the shoot tip; 1 closed, 3 half open, 5 fully open.	5	5	5	5
OIV 003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	1	7	1	5
OIV 004	Young shoot: density of prostrate hairs on the shoot tip; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	5	7	5
OIV 006	Shoot: attitude (before tying); 1 erect, 3 semi-erect, 5 horizontal, 7 semi-drooping, 9 drooping.	1	3	1	3
OIV 007	Shoot: color of the dorsal side of internodes; 1 green, 2 green and red, 3 red.	1	2	1	2
OIV 008	Shoot: color of the ventral side of internodes; 1 green, 2 green and red, 3 red.	1	2	1	2
OIV 015-2	Shoot: intensity of anthocyanin coloration on the bud scales; 1 none or very weak, 3 weak, 5 medium, 7 strong, 9 very strong.	1	5	1	3
OIV 016	Shoot: number of consecutive tendrils; 1 two or less, 2 three or more.	1	1	1	1
OIV 051	Young leaf: color of upper side of blade (4th leaf); 1 green, 2 yellow, 3 bronze, 4 copper-reddish.	1	3	3	3
OIV 053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf); 1 none or very low, 3 low, 5, medium, 7 high, 9 very high.	7	7	9	5
OIV 065	Mature leaf: size of blade; 1 very small, 3, small, 5 medium, 7 large, 9 very large.	5	5	5	7
OIV 067	Mature leaf: shape of blade; 1 cordate, 3 wedge-shaped, 3 pentagonal, 4 circular, 5 kidney-shaped.	3	3	3	3
OIV 068	Mature leaf: number of lobes; 1 one, 2 three, 3 five, 4 seven, 5 more than seven.	3	3	3	3
OIV 070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade; 1 absent, 2 only at the petiolar point, 3 up to the 1st bifurcation, 4 up to the 2nd bifurcation, 5 beyond the 2nd bifurcation.	1	1	1	3
OIV 074	Mature leaf: profile of blade in cross section; 1 flat, 2 V-shaped, 3 involute, 4 revolute, 5 twisted.	5	5	3	4
OIV 075	Mature leaf: blistering of upper side of blade; 1 absent or very weak, 2 weak, 3 medium, 4 strong, 9 very strong.	5	3	5	3
OIV 076	Mature leaf: shape of teeth; 1 both sides concave, 2 both sides straight, 3 both sides convex, 4 one side concave on side convex, 5 mixture between both sides straight and both sides convex.	3	3	2	3
OIV 079	Mature leaf: degree of opening/overlapping of petiole sinus; 1 very wide open, 3 open, 5 closed, 7 overlapped, 9 strongly overlapped.	7	7	3	5
OIV 080	Mature leaf: shape of base petiole sinus; 1 U-shaped, 2 brace-shaped, 3 V-shaped.	3	3	3	3
OIV 081-1	Mature leaf: teeth in the petiole sinus; 1 none, 9 present.	1	2	1	1
OIV 081-2	Mature leaf: petiole sinus base limited by vein; 1 not limited, 3 on one side, 3 on both sides.	1	1	1	1

Table 2. *Cont.*

Code	Descriptor	CÑ	CS	MP	PF
OIV 083-1	Mature leaf: shape of the base of upper lateral sinuses; 1 U-shaped, 2 brace-shaped, 3 V-shaped.	3	3	1	1
OIV 083-2	Mature leaf: teeth in the upper lateral sinuses; 1 none, 9 present.	1	1	1	1
OIV 084	Mature leaf: density of prostrate hairs between main veins on lower side of blade; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	7	5	7
OIV 085	Mature leaf: density of erect hairs between main veins on lower side of blade; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	5	5	5
OIV 086	Mature leaf: density of prostrate hairs on main veins on lower side of blade; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	5	9	5
OIV 087	Mature leaf: density of erect hairs on main veins on lower side of blade; 1 none or very low, 3 low, 5 medium, 7 high, 9 very high.	5	5	3	1
OIV 151	Flower: sexual organs; 1 fully developed stamens and no gynoecium, 2 fully developed stamens and reduced gynoecium, 3 fully developed stamens and fully developed gynoecium, 4 reflexed stamens and fully developed gynoecium.	3	3	3	3
OIV 202	Bunch: length (peduncle excluded); 1 very short, 3 short, 5 medium, 7 long, 9 very long.	7	7	7	7
OIV 203	Bunch: width; 1 very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide.	5	5	5	5
OIV 204	Bunch: density; 1 very loose, 3 loose, 5 medium, 7 dense, 9 very dense.	3	5	5	5
OIV 206	Bunch: length of peduncle of primary bunch; 1 very short, 3 short, 5 medium, 7 long, 9 very long.	1	1	1	1
OIV 220	Berry: length; 1 very short, 3 short, 5 medium, 7 long, 9 very long.	5	5	5	3
OIV 221	Berry: width; 1 very narrow, 3 narrow, 5 medium, 7 wide, 9 very wide.	5	5	5	3
OIV 223	Berry: shape; 1 obloid, 2 globose, 3 broad ellipsoid, 4 narrow ellipsoid, 5 cylindric, 6 obtuse ovoid, 7 ovoid, 8 obovoid, 9 horn shaped, 10 finger shaped.	1	7	5	2
OIV 225	Berry: color of skin; 1 green yellow, 2 rose, 3 red, 4 grey, 5 dark red violet, 6 blue black.	1	1	1	1

CÑ: Cañocazo. CS: Castellano. MP: Mantúo de Pilas. PF: Palomino Fino.

3.3. Grape Must Physicochemical Characterization

Table 3 shows the results (mean value ± standard deviation) of the physicochemical analyses carried out on the musts from the four varieties studied over two consecutive years. The autochthonous varieties showed differences in sugar concentration in the two years studied.

The pH values obtained for the four varieties studied were high and very similar for the two years of the study, with slightly higher values observed for the Castellano and Mantúo de Pilas varieties, regardless of the year. In terms of organic acid content, tartaric acid represented more than 70% of the total acidity of the musts from all the varieties. Their values did not vary significantly from one year to the next regardless of the degree of ripeness, but their concentration was always higher in the autochthonous varieties than in the control variety (Palomino Fino). On the other hand, the malic acid content varied from one year to the next, especially in the autochthonous varieties (CÑ, CS, and MP). In the case of these varieties, malic acid content was higher in 2016, when higher Baume degrees and ripening index were reached. As for the oxidative index, or tendency of the musts to enzymatic oxidation, the musts of the varieties analyzed presented differences, and their values did not generally differ between the two years studied. In both years, Palomino Fino grape musts presented a tendency to oxidation higher than the rest of the autochthonous varieties, being the lowest results showed by Cañocazo. Yeast assimilable nitrogen (YAN) content differed between the varieties studied, reaching higher values in 2016. As with the oxidative index, the Palomino Fino grape must had the highest YAN values in both years. Regarding the concentration of the different cations analyzed, the different varieties showed differences in cation content, maintaining these differences during the two years of study. Potassium was the predominant cation, followed by calcium, and magnesium.

Table 3. Autochthonous varieties and Palomino Fino grape must characterization during two consecutive years (2016 and 2017).

Physicochemical Parameters	CÑ			CS			MP			PF		
2016												
°Bé	10.88	±	0.02 ^{a}	11.54	±	0.01 ^{b}	9.12	±	0.02 ^{c}	11.10	±	0.01 ^{d}
pH	3.72	±	0.02 ^{a}	3.94	±	0.01 ^{b}	3.93	±	0.07 ^{b}	3.77	±	0.01 ^{a}
TA ($g{\cdot}L^{-1}$ TH_2)	3.79	±	0.06 ^{a}	3.47	±	0.04 ^{b}	3.51	±	0.07 ^{b}	3.24	±	0.06 ^{c}
Ripening Index (RI)	2.87	±	0.02 ^{a}	3.32	±	0.06 ^{b}	3.51	±	0.01 ^{c}	3.42	±	0.04 ^{b}
Tartaric acid (g/L)	2.80	±	0.03 ^{a}	2.96	±	0.01 ^{b}	2.88	±	0.05 ^{a}	2.37	±	0.04 ^{c}
Malic acid(g/L)	1.08	±	0.08 ^{a}	0.80	±	0.03 ^{b}	1.20	±	0.10 ^{c}	0.24	±	0.00 ^{d}
Glycerin (g/L)	0.26	±	0.00 ^{a}	0.35	±	0.00 ^{b}	0.33	±	0.00 ^{b}	0.19	±	0.01 ^{d}
Oxidative index (%)	10.45	±	0.64 ^{a}	29.97	±	1.05 ^{b}	21.18	±	0.71 ^{c}	56.32	±	1.57 ^{d}
YAN (mg/L)	184.73	±	1,07 ^{a}	188.50	±	1.54 ^{a,c}	143.57	±	2.50 ^{b}	192.57	±	2.14 ^{c}
Calcium (mg/L)	362.33	±	1.00 ^{a}	158.70	±	1.00 ^{b}	146.07	±	1.00 ^{c}	167.95	±	1.00 ^{b}
Magnesium (mg/L)	151.61	±	0.30 ^{a}	82.72	±	0.20 ^{b}	80.72	±	0.40 ^{b}	75.47	±	0.20 ^{c}
Sodium (mg/L)	13.16	±	0.20 ^{a}	9.24	±	0.11 ^{b}	6.06	±	0.40 ^{c}	13.82	±	0.50 ^{d}
Potassium (mg/L)	3228.55	±	4.00 ^{a}	2369.66	±	9.01 ^{b}	1749.59	±	9.00 ^{c}	2308.84	±	12.00 ^{b}
Iron (mg/L)	3.79	±	0.01 ^{a}	6.21	±	0.04 ^{b}	6.25	±	0.10 ^{c}	8.20	±	0.20 ^{d}
Copper (mg/L)	0.79	±	0.01 ^{a}	1.20	±	0.02 ^{b}	1.19	±	0.04 ^{b}	3.95	±	0.03 ^{c}
2017												
°Bé	9.05	±	0.02 ^{a}	9.40	±	0.01 ^{b}	8.65	±	0.01 ^{c}	10.65	±	0.01 ^{d}
pH	3.76	±	0.01 ^{a}	3.92	±	0.01 ^{b}	3.90	±	0.01 ^{b}	3.89	±	0.01 ^{b}
TA ($g{\cdot}L^{-1}$ TH_2)	3.82	±	0.02 ^{a}	3.56	±	0.01 ^{b}	3.53	±	0.01 ^{b}	3.46	±	0.01 ^{b}
Ripening Index (RI)	2.37	±	0.02 ^{a}	2.64	±	0.02 ^{b}	2.45	±	0.10 ^{a}	3.07	±	0.04 ^{c}
Tartaric acid (g/L)	2.80	±	0.01 ^{a,c}	2.90	±	0.01 ^{b}	2.68	±	0.01 ^{a,b}	2.58	±	0.01 ^{c}
Malic acid(g/L)	0.37	±	0.01 ^{a}	0.34	±	0.01 ^{b}	0.46	±	0.01 ^{c}	0.31	±	0.01 ^{d}
Glycerin (g/L)	0.03	±	0.01 ^{a}	1.30	±	0.01 ^{b}	0.21	±	0.01 ^{c}	0.03	±	0.01 ^{a}
Oxidative index (%)	9.46	±	0.10 ^{a}	27.49	±	0.97 ^{b}	19.98	±	0.89 ^{c}	50.24	±	1.42 ^{d}
YAN (mg/L)	168.24	±	0.98 ^{a}	175.73	±	1.24 ^{b}	140.30	±	2.80 ^{c}	184.24	±	1.70 ^{d}
Calcium (mg/L)	371.22	±	0.98 ^{a}	159.22	±	1.14 ^{b}	154.28	±	1.03 ^{b}	179.91	±	2.05 ^{c}
Magnesium (mg/L)	148.72	±	0.41 ^{a}	78.74	±	0.15 ^{b}	79.70	±	1.22 ^{b}	71.52	±	0.18 ^{c}
Sodium (mg/L)	13.89	±	0.18 ^{a}	9.01	±	0.09 ^{b}	6.37	±	0.34 ^{c}	12.87	±	0.38 ^{d}
Potassium (mg/L)	3105.28	±	7.06 ^{a}	2472.02	±	6.97 ^{b}	1821.46	±	11.06 ^{c}	2340.51	±	8.85 ^{d}
Iron (mg/L)	4.02	±	0.01 ^{a}	6.23	±	0.01 ^{b}	6.21	±	0.08 ^{b}	7.98	±	0.07 ^{c}
Copper (mg/L)	0.82	±	0.02 ^{a}	0.99	±	0.03 ^{b}	1.27	±	0.03 ^{c}	4.11	±	0.14 ^{d}

Different superscript letters mean statistically significant differences between samples at p-adjust < 0.05 obtained by one-way ANOVA and Bonferroni's multiple range (BSD) test. Results are the means ± SD of three repetitions. CÑ: Cañocazo. CS: Castellano. MP: Mantúo de Pilas. PF: Palomino Fino.

The results of the principal component analysis (PCA) (Figure 1) based on the physicochemical data of the different varieties, showed two factors that explain 76.7% of the total variance. Factor 1 (F1), representing 44.7% of the variance, correlates positively with the total acidity, tartaric, and malic acid, and with the main cations in musts (potassium, calcium, and magnesium), and negatively with the pH, the oxidative index, and the metallic cations iron and copper. Factor 2 (F2), which explains 32.02% of the variance, correlates positively with density, YAN, and cations potassium and sodium, and negatively with pH, tartaric acid, and malic acid. As can be seen, the representation of the values leads to a segregation of the different varieties independently of the year of study. Of the two factors obtained, F2 is the one that discriminates the most between the varieties. F1 is higher in all the autochthonous varieties studied, with respect to Palomino Fino, highlighting Cañocazo. The varieties Mantúo de Pilas and Castellano presented similar values of F1 and values closer to Palomino Fino. On the other hand, Palomino Fino has the highest F2 values, followed by Castellano, Cañocazo, and finally Mantúo de Pilas. This corresponds to the values of the ripening index (°Bé/total acidity) that were calculated for the different varieties (Table 3).

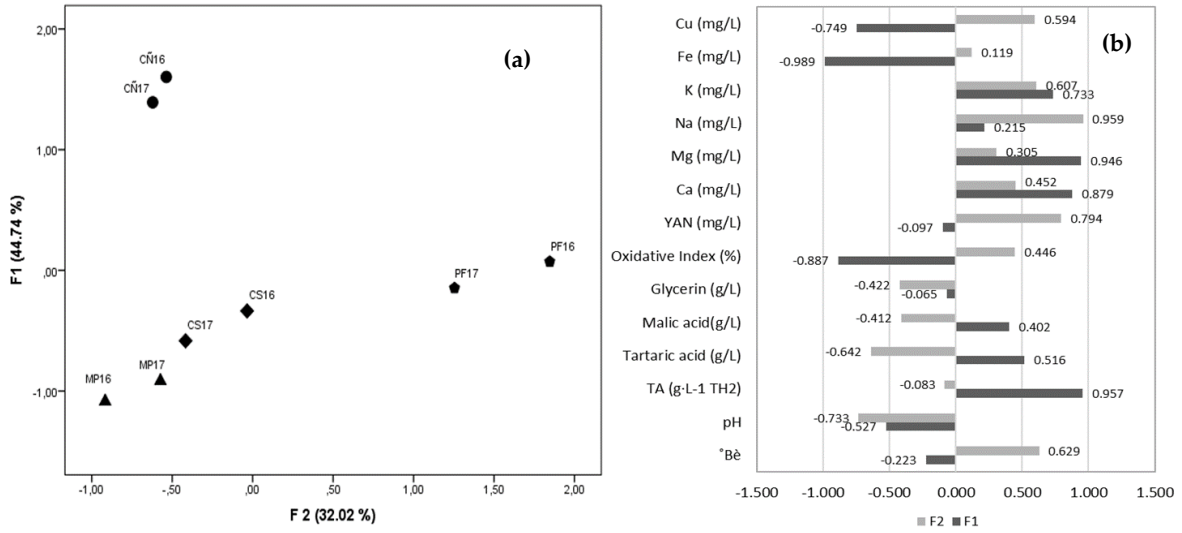

Figure 1. Principal component analysis (**a**) and its loading factors (**b**) of Cañocazo [CÑ], Castellano [CS], Mantúo de Pilas [MP], and Palomino Fino [PF] grape musts physicochemical analysis during two consecutive years (2016 and 2017).

4. Discussion

The use of SSR molecular markers is recommended for the genetic identification of vine varieties [33]. The analysis of several microsatellites allows a unique fingerprint to be obtained for each variety [34]. However, it is very important that the same set of microsatellites is used in this work in order to allow comparison of the genotypes obtained with those in other databases. There is also an international consensus that six microsatellite loci are the minimum number that can be used to discriminate between two varieties [24]. In the case of closely related varieties, the number of these should be increased [35]. In this study, a set of 22 microsatellite loci has been analyzed, consisting of the six recommended by the OIV, and agreed upon as a result of the GENRES 081 project (VVMD5, VVMD7, VVMD27, VVS2, VrZAG62, and VrZAG79). This has been extended to 22 with those proposed by the European GrapeGen06 project. After comparing the genotypes obtained for the autochthonous varieties with the *Vitis* International Variety Catalogue (*V*IVC) database, two synonyms that were previously described in this database have been confirmed for Castellano and Mantúo de Pilas. The genotype presented by the variety Castellano corresponds to that of Manteudo for the nine microsatellite loci (*V*IVC), and 20 loci, according to Lacombe et al. [23]. This variety is registered as a white variety autochthonous to Portugal, and is conserved in Spain under this name only at Finca El Encín (Holding Institution Code: ESP080). In this study, the genotype is extended by 13 additional loci.

The variety Mantúo de Pilas has presented the same genotype as the variety De Rey; thus, confirming the synonymy described by Sancho-Galán et al. [21] for the 22 microsatellite loci studied.

Alongside the genetic characterization, a morphological characterization was carried out in order to obtain a complete description of the vine material [36]. The varieties Cañocazo and Palomino Fino have shown a similar phenotype to the one described by García de Lujan et al. [37], while the variety Castellano is similar to that described by Serrano et al. [38]. However, the variety Mantúo de Pilas analyzed in this work, differs slightly with respect to its phenotype in some of the descriptors, when compared to those described for Uva Rey, which had been confirmed as synonyms through genetic identification [21]. These differences could be attributed to environmental conditions, since the two varieties that have been compared are planted in plots of land at different geographical locations. All of the autochthonous varieties presented a medium to high density of hairiness on adult leaves (OIV 084, OIV 085, OIV 086, and OIV 087), similar to Palomino Fino. Non-glandular vine hairs or trichomes play a functional role in the plant since they modulate evapotranspiration by restricting air movement around the stomata pores [39]. Thus, all the varieties that were studied could be considered as autochthonous white varieties adapted to warm climate areas because of their higher hair density.

In recent years, we have seen changes in vineyard development, such as premature budding and flowering as a result of higher temperatures, and changes in the rainfall regime associated with global climate warming [40]. These changes have the potential to affect the concentration of the different secondary metabolites in berries since they are mostly influenced by the physiological activity of the vines during the grape ripening stage (from July to September in Marco de Jerez region). Latterly, the grape ripening stage has been affected by climate change, and the vegetative period in which the plant carries out physiological activities has been lengthened [40]. This has increased metabolic rates in vineyards, and therefore affected the secondary metabolites content in berries and consequently in their musts [41,42]. After studying two consecutive years, it has become clear how global warming could affect autochthonous grape musts composition, particularly with regard to the balance between sugar content and malic acid concentration; an increase in sugar content is accompanied by a decrease in malic acid concentration in berries during the ripening stage [43]. The accumulation of sugars in berries takes place because of the mobilization of reserves in stems and roots, and of sugars from leaf photosynthesis [44]; the malic acid is then consumed in the grain cell by respiratory combustion as a substrate energy source [45]. Some studies have shown that malic acid consumption is enhanced when the weather is warmer during the ripening stage [45,46]. This is why low levels of malic acid in grape musts are common in warm climate areas [47,48]. Analyzing the evolution of temperatures during the years 2016 and 2017 (Figures S1 and S2), it may be thought that in the year with the hottest ripening period (2016), musts with a higher °Bé and lower malic acid content than in 2017 should be obtained. This is true for the Palomino Fino variety (Table 3); however, with the other autochthonous varieties the opposite phenomenon occurs. This lack of correlation between these two parameters in autochthonous varieties might be due to a ripening problem because of the high temperatures. The temperatures reached in 2016 were very high, punctually (Figure S1), and this, together with a low rainfall, could have led to a biological and metabolic interruption in the grape cells, as well as an increase in the final density of the grapes, mainly due to the phenomena of water evaporation. Nonetheless, the year 2017 (Figure S2), with relatively lower temperatures, was more favorable to the biological activity of the autochthonous varieties, and grape musts with lower malic acid content were obtained.

Between varieties, Castellano and Palomino Fino showed a higher concentration of sugars. This may be due to the fact that these varieties have a shorter phenological cycle than Mantúo de Pilas and Cañocazo [37]; therefore, they ripen earlier, causing a lower concentration of sugars in berries. The choice of the variety, according to the climate, is a matter of great importance in order to obtain ripe grapes with a balanced composition. According to Hidalgo-Togores [44], in warm climates, varieties with a late cycle should be used so that the grapes mature when the climate is more favorable. In this sense, it is reasonable to think that the autochthonous varieties, especially Mantúo de Pilas and Cañocazo, should be harvested later than Palomino Fino.

In addition to the organic acids, YAN content and the oxidative index of grape musts could also be considered as characteristics of the variety, although they are subject to fluctuations, depending on the year and the environmental conditions during the ripening period. The differences found in YAN values between the varieties could also be due to differences in the degree of ripeness as the YAN content increases during grape ripening [49]. In spite of that, the time lag in the ripening cycle between the varieties, all of them reached levels higher than 140 mg/L; thus, ensuring the proper development of alcoholic fermentation [50].

With respect to the grape musts oxidative index, Palomino Fino—from both years—shows a greater tendency to oxidize. This fact could be because this variety generally presents a very high content in polyphenolic compounds susceptible of being oxidized [51]. A greater presence of iron and copper, which are powerful catalysts for this reaction, may also contribute to the oxidation of polyphenols by chemical means [52]. The quantification of the remaining cations is of major importance for wines since they may exert physiological effects on the consumer or hinder technological processes, such as wine stabilization [44]. With regard to the other cations found in the samples, it was observed that potassium represents almost the entire concentration of those cations, since it is the most important ionic compound present in grapes, and plays a major role in the enzymatic reactions and processes of grapes [44,53]. It is important to determine calcium and magnesium content, since the former, similar to potassium, may cause precipitation problems in wines (calcium tartrate). However, the concentration of both cations is highly influenced by the geographical area of origin of the grapes and the composition of soil [54]. Finally, sodium content is significantly below the limit established by the OIV, and this cation does not pose a problem for wine production or consumption [28].

The PCA, together with all of the physicochemical variables analyzed, corroborates the results and the differences determined between the varieties in this research. On the one hand, F1 (Figure 1), establishes that autochthonous varieties studied could have a higher acidity potential than Palomino Fino, regardless of the year or ripeness level; being Cañocazo particularly noteworthy. This fact, combined with warm climate conditions, constitutes an advantage when it comes to making white wines with an improved sweetness/acidity balance. The main cations found in grape musts (potassium, magnesium, and calcium), with positive loading factors (Figure 1), also contribute significantly to F1, while metals (iron and copper), with negative values, also make a considerable contribution (Table 3). Therefore, the high acidity factor of Cañocazo is due partly to the fact that its must has significantly higher levels of potassium, magnesium, and calcium and lower levels of iron and copper than the other varieties. F2, which is positively correlated with grape must density, can clearly discriminate between all the varieties that have been studied, regardless of the year (Figure 1). The lower values of F2 in the autochthonous varieties would corroborate that they have been harvested earlier and require longer ripening periods, especially Mantúo de Pilas, since it has longer cycles than Palomino Fino.

5. Conclusions

Molecular analysis with 22 SSR loci allowed the identification of autochthonous varieties with different genotypes. However, all of them showed similar phenotypic characteristics in terms of high hair density on adult leaves, which could be of interest as a mechanism to regulate grapevine evapotranspiration, and therefore adapt to an increase in temperature as a consequence of global warming. With regard to the physicochemical composition of the musts, after multivariate analysis of the results, different behaviors have been observed among the autochthonous varieties, with respect to the control variety Palomino Fino. It should be highlighted that Mantúo de Pilas and Cañocazo had a longer phenological cycle and, as a result, a higher acidity, thereby allowing for the production of quality wines in hot climate areas. As a result of all the above, these autochthonous varieties could be considered suitable for cultivation in areas with warmer and drier climates, a trend that has been observed in many winemaking regions as a consequence of the climate change. In order to promote their cultivation, it would be necessary to apply for their inclusion in the Official Register of Authorized Varieties.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/2/205/s1, Figure S1: (a) Temperature (°C) (T_max, T_min, T_avg), (b) humidity (%) (H_max, H_min, H_avg), and (c) radiation (W/m^2) and rainfall (L/m^2) among July and September 2016. Figure S2: (a) Temperature (°C) (T_max, T_min, T_avg), (b) humidity (%) (H_max, H_min, H_avg), and (c) radiation (W/m^2) and rainfall (L/m^2) among July and September 2017.

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